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initial methionine residue), and the standard one-letter abbreviation for amino acids is used. --

Please replace the paragraph beginning at page 4, line 12, with the following written paragraph:

-- Figures 2A-B show the cDNA and corresponding deduced amino acid sequence of ICE-LAP-4. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue). --

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Please replace the paragraph beginning at page 4, line 16, with the following written paragraph:

-- Figures 3A-C show an amino acid sequence comparison between ICE-LAP-3, ICE-LAP-4, human ICE and the *C. elegans* cell death gene ced-3. Shaded areas represent amino acid matches between the different sequences. --

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Please replace the paragraph beginning at page 4, line 20, with the following written paragraph:

-- Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figures 1A-B and 2A-B are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%. --

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Please replace the paragraph beginning at page 4, line 25, with the following written paragraph:

-- In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode the mature polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or for the mature polypeptide encoded by the cDNA of the clones deposited as ATCC Deposit No. 75875 encoding ICE-LAP-3, and ATCC Deposit No. 75873 encoding ICE-LAP-4, which were deposited August 25, 1994. --

Please insert the following paragraph on page 4, after line 32:

--These deposits are biological deposits with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209. Since the strains referred to are being maintained under the terms of the Budapest Treaty, they will be made available to a patent office signatory to the Budapest Treaty.--

Please replace the paragraph beginning at page 4, line 33, with the following written paragraph:

-- The polynucleotide encoding ICE-LAP-3 can be detected from human prostate, human endometrial tumor, human pancreatic tumor, human adrenal gland tumor and human tonsil. The full-length encoding ICE-LAP-3 was discovered in a cDNA library derived from human endometrial tumor. It is structurally related to the Interleukin-1 converting enzyme family. It contains an open reading frame encoding a protein of approximately 341 amino acid residues. The protein exhibits the highest degree of homology to *C. elegans* cell death gene ced-3 which is a homolog of human interleukin-1 converting enzyme, with 68 % similarity and 43 % identity over the entire amino acid sequence. It should be pointed out that the pentapeptide QACRG is conserved and is located at amino acid position 184-188. --

Please replace the paragraph beginning at page 5, line 21, with the following written paragraph:

-- The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encode the mature polypeptides may be identical to the coding sequence shown in Figures 1A-B and 2A-B or that of the deposited clones or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encode the same mature polypeptides, and derivatives thereof, as the DNA of Figures 1A-B and 2A-B or the deposited cDNA. --

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Please replace the paragraph beginning at page 5, line 33, with the following written paragraph:

-- The polynucleotides which encode for the mature polypeptides of Figures 1A-B and 2A-B or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide. --

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Please replace the paragraph beginning at page 6, line 10, with the following written paragraph:

-- The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be naturally occurring allelic variants of the polynucleotides or non-naturally occurring variants of the polynucleotides. --

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Please replace the paragraph beginning at page 6, line 18, with the following written paragraph:

-- Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1A-B and 2A-B or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-B or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants. --

Please replace the paragraph beginning at page 6, line 27, with the following written paragraph:

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-- As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figures 1A-B and 2A-B or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of nucleotides, which does not substantially alter the function of the encoded polypeptides. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. --

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Please replace the paragraph beginning at page 7, line 11, with the following written paragraph:

-- The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNAs of Figures 1A-B and 2A-B or the deposited cDNA(s). --

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Please replace the paragraph beginning at page 8, line 4, with the following written paragraph:

-- The present invention further relates to ICE-LAP-3 and 4 polypeptides which have the deduced amino acid sequence of Figures 1A-B and 2A-B or which has the amino acid sequence encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptides. --

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Please replace the paragraph beginning at page 8, line 9, with the following written paragraph:

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-- The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1A-B and 2A-B or that encoded by the deposited cDNA, means polypeptides which retain essentially the same biological function or activity as such polypeptides, and wherein derivatives include polypeptides with enhanced or reduced biological function. An analog includes a proprotein portion to produce active mature polypeptides. --

Please replace the paragraph beginning at page 8, line 20, with the following written paragraph:

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-- The fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-C or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the mature polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein. --

Please replace the paragraph beginning at page 26, line 11, with the following written paragraph:

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--The DNA sequence encoding ICE-LAP-3, ATCC # 75875, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-3 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-3 gene. Additional nucleotides corresponding to ICE-LAP-3 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGCGTGC~~GGGG~~ACACGGGTC 3' (SEQ ID NO:5) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5'

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GTACTCTAGATCATTCACCCTGGTGGAGGAT 3' (SEQ ID NO:6) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-3. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-3 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-3 (95% pure is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar gluathione (reduced) and 2 mmolar glutathione (oxidized). After

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incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.--

Please replace the paragraph beginning at page 28, line 1, with the following written paragraph:

-- The DNA sequence encoding ICE-LAP-4, ATCC # 75873, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-4 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-4 gene. Additional nucleotides corresponding to ICE-LAP-4 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGGAGAACACTGAAAACTCA 3' (SEQ ID NO:7) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATTAGTGATAAAAATAGAGTTC 3' (SEQ ID NO:8) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-4. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). PQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp

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(100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ration of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-4 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-4 (95% pure is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar gluathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.--

Please replace the paragraph beginning at page 30, line 7, with the following written paragraph:

-- The DNA sequence encoding for ICE-LAP-3, ATCC # 75875, was constructed by PCR on the full-length ICE-LAP-3 using two primers: the 5' primer 5' GACTATGCGTGCAGGGACACGG 3' (SEQ ID NO:9) contains the ICE-LAP-3 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-3 coding sequence starting from the initiation codon; the 3' sequence

5'

AATCAAGCGTAGTCTGGGACGTCGTATGGGTATTCACCTGGTGGAGGATTG 3' (SEQ ID NO:10) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-3 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-3 coding sequence followed by HA tag fused in frame, and a translation termination stop codon next to the HA tag. The PCR amplified DNA fragment was ligated with pcDNA1/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the

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transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-3, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-3 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

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Page 31, line 6, after "SDS-PAGE gels." delete the remainder of the page which is empty space.

Please replace the paragraph beginning at page 32, line 20, with the following written paragraph:

-- The DNA sequence encoding for ICE-LAP-4, ATCC # 75873, was constructed by PCR on the full-length ICE-LAP-4 using two primers: the 5' primer 5' ACCATGGAGAACACTGAAAAC 3' (SEQ ID NO:11) contains the ICE-LAP-4 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-4 coding sequence starting from the initiation codon; the 3' sequence

5'

AATCAAGCGTAGTCTGGGACGTCGGTATGGGTAGTGATAAAATA GAGTTCTTT 3' (SEQ ID NO:12) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-4 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-4 coding sequence followed by HA tag fused in frame, and a translation termination stop codon next to the HA tag. The PCR amplified DNA fragment was ligated with pcDNA1/Amp by blunt end ligation. The ligation mixture was

transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-4, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-4 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with 35 S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

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Please replace the sequence listing at pages 35-41 of the specification with the Substitute Sequence Listing (pages 1-6) attached hereto.

In the Figures:

Please cancel Figure 1, without prejudice, and replace it with new Figures 1A-B, submitted herewith with the formal drawings.

Please cancel Figure 2, without prejudice, and replace it with new Figures 2A-B, submitted herewith with the formal drawings.

Please cancel Figure 3, without prejudice, and replace it with new Figures 3A-C, submitted herewith with the formal drawings.